

Recent observations on the serology of syphilis*

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SUMMARY Routine screening of 404 742 sera by the automated micro-haemagglutination assay (AMHA-TP) and the Venereal Disease Research Laboratory (VDRL) test showed that 9848 specimens gave a reactive result to one of the three assays. Reactive results were confirmed by the fluorescent treponemal antibody absorption (FTA-ABS) test. The possibility of false-positive results varied from 0.04-0.38% of all specimens or from 1.7-15.7% of reactive sera. The VDRL test failed to detect reactivity in 56.54% of sera from patients who had previously been infected with *Treponema pallidum*.

The importance of routine testing by the AMHA-TP is illustrated by the detection of four patients with mesaortitis and two with active neurosyphilis among a selected group of 54 patients who had non-reactive results to the VDRL test. Testing of cerebrospinal fluid specimens by the AMHA-TP test produced more specific results than by the other two tests.

Introduction

Recent research into the serology of syphilis has concentrated on the identification of specific IgM and IgG antibodies against *Treponema pallidum*. The basic fluorescent treponemal antibody absorption (FTA-ABS) test however allows a margin of error¹⁻⁵ which may be 6.8-10.7%,⁶ 7%,⁷ or 37.2%.⁸ The sources of error may be non-specific group antibodies, the use of an inefficient sorbent, changes on the cell surface due to lyophilising of *T. pallidum*, and to other factors.^{4 9-12}

The occurrence of *T. pallidum*-specific antibodies of the IgM class seems to indicate early or active syphilitic infection whereas their disappearance after antibiotic treatment may prove a cure.^{10 11 13-16}

The methods used in these investigations are so far not precise enough for definite conclusions.^{11 17 18} An improvement in methods is taking place in several research centres and promises a better approach in a few years.

The results of the *T. pallidum* haemagglutination assay (TPHA) have been assessed by many workers in the last few years.¹⁹⁻³⁷ This test appears to be the

most sensitive, specific, and economical method for the serodiagnosis of syphilis. It indicates the presence of IgM and IgG antibodies against *T. pallidum* and may therefore become reactive before any other test.^{23-25 37}

The micro-haemagglutination assay (MH-TP) and especially the automated version of this procedure (AMHA-TP) is most valuable for screening purposes.^{12 19 23-25 27 38-43} The common trend in screening is towards the application of the AMHA-TP and a lipoidal antigen test, the Venereal Disease Research Laboratory (VDRL) test, the rapid plasma reagin (RPR) test,^{24 27 40} the reagin serum (RST) test,⁴³ and others. At present however the VDRL test is the best standardised test in use based on 25 years' experience.

Methods

The use of the AMHA-TP test and the VDRL test for screening was first suggested in 1972 by Luger and Spendlingwimmer.²⁵ Since that time, 404 742 sera have been examined during a period of six years at the Department of Dermatology in the City Hospital Vienna-Lainz and evaluated by the Ludwig-Boltzmann Institute of Dermatovenereological Serodiagnosis. All samples were received from hospitals, outpatient dispensaries, or the central health office in Vienna. The sera were tested routinely by the AMHA-TP and by the VDRL test. Reactive results were checked by the FTA-ABS test. The tests were performed as described.^{10 24 27}

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Results and comments

The results of sera examined between 13 March 1972 and 30 April 1978, as well as of the reactivity to the three tests, are given in Table I. A total of 394 894 samples were non-reactive to the AMHA-TP and to the VDRL test and were therefore not investigated further. Of the remainder, 9848 sera were reactive to one or both of the two screening tests and the results were checked by the FTA-ABS test.

TABLE I Results of serological tests on 404 742 sera

Reactivity to following tests			No of sera	% of total No reactive to 1/3 tests	% of total No examined
AMHA-TP	FTA-ABS	VDRL			
+	-	-	1377	13.98	0.34
+	-	-	4226	42.91	1.04
+	+	+	3248	32.98	0.80
+	-	+	95	0.97	0.02
-	+	+	73	0.74	0.02
-	-	+	829	8.42	0.21
Total No reactive to 1/3 tests			9848	100.00	2.43
Total No non-reactive to AMHA-TP/VDRL			394 849		97.57
Grand total			404 742		100.00

+ Reactive - non-reactive

Of the 1377 sera showing reactive results to the AMHA-TP but non-reactive results to the FTA-ABS test and the VDRL test, a few may have occurred in patients at the beginning of the infection (before the third week) but, as far as could be traced, they were mainly found in patients who had contracted syphilis many years previously, were adequately treated, and were probably cured. Some sera came from old people who denied having had syphilis.

No history was obtainable from most of the donors. Further investigations to try to elucidate the reasons for an isolated reactive result to the AMHA-TP were undertaken but these results could have been non-specific (false-positive results) in several cases.

The results of the 4226 specimens which were reactive to the AMHA-TP and to the FTA-ABS test but not to the VDRL test indicate that the donors had syphilis, but the non-reactivity of the VDRL test suggests that the infection had occurred many years previously, had been treated successfully, or had disappeared spontaneously.

Since 3248 sera were reactive to the AMHA-TP, to the FTA-ABS, and to the VDRL tests, there can be no doubt the donors all had syphilis. Ninety-five specimens were reactive to the AMHA-TP and to the VDRL test but non-reactive to the FTA-ABS test, and 73 specimens were non-reactive to the AMHA-TP test but reactive to the FTA-ABS and VDRL

tests. These latter two groups comprise results which showed either false-reactivity or false non-reactivity in one of the two tests using treponemal antigens. The results for the 829 sera which were non-reactive to the AMHA-TP and the FTA-ABS test but reactive to the VDRL test can be considered biological false-positive reactions.

The possibility of obtaining false results by the above method of screening may vary from 1.7 to 15.7% of the reactive samples and from 0.04 to 0.38% of all sera examined. The first figure constitutes an absolute minimum, the last an improbable maximum. The rate of specific reactivity of 1.84% (minimum) seems to be high but this is due to the selection of patients for serum antibody tests in most of the hospitals and dispensaries.

NON-REACTIVE RESULTS IN SELECTED GROUP

Results were obtained from 53 patients who were reactive to the AMHA-TP as well as to the FTA-ABS tests but non-reactive to the VDRL test. These patients were asked to attend for clinical examination. Of these, 10 had a history of a previous infection and had been adequately treated. Of the 43 who did not know that they had once had syphilis, 37 had no symptoms, four had mesaortitis with aortic diameters ranging from 40 to 50 mm on their radiographs (only one had attended the hospital because of cardiac complaints, one attended because of a leg ulcer, one had hepatic cirrhosis, and one had a keratoacanthoma), and two had changes of active neurosyphilis in the cerebrospinal fluid (one had tabes dorsalis and the other, clinically asymptomatic meningovascular syphilis with focal signs on electroencephalographs). Both these latter patients attended the department of urology, the first because he had difficulties of micturition (early tabetic paralysis of the bladder) and the other because of vague urological complaints. Late syphilis was discovered in all six patients by routine serum antibody tests.

These investigations could be performed on only 53 (1.25%) of 4226 patients and concern exclusively patients of the City Hospital, Lainz. These observations were therefore of a highly selected group and are not at all representative, although the results emphasise the importance of routine serological testing with the AMHA-TP instead of the VDRL test.

COMPARATIVE EVALUATION OF TESTS

In a comparative evaluation of the three tests (Table II), the agreement between the AMHA-TP and FTA-ABS test was 82.87% of the reactive samples or 99.61% of all sera (reactive plus non-reactive results); agreement between the AMHA-TP and the VDRL test was 33.95% or 98.39% respectively and

TABLE II Comparative evaluation of results to three tests

Reactivity to following tests	Reactivity to following tests											
	FTA-ABS						VDRL					
	-		+		Total		-		+		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
VDRL												
-			4226	49.89	4226	49.89						
+	924	10.91	3321	39.20	4245	50.11						
Total	924	10.91	7547	89.09	8471	100.00						
AMHA-TP												
-			73	0.81	73	0.81			902	9.15	902	9.15
+	1472	16.32	7474	82.87	8946	99.19	5603	56.90	3343	33.95	8946	90.85
Total	1472	16.32	7547	83.68	9019	100.00	5603	56.90	4245	43.10	9848	100.00

+ Reactive - non-reactive

between the FTA-ABS test and the VDRL test 39.2% or 98.72% respectively. The somewhat better agreement between the FTA-ABS and VDRL tests compared with the AMHA-TP and VDRL test can be explained by the fact that the FTA-ABS test sometimes (in primary and in early secondary syphilis) returns earlier to non-reactivity after adequate treatment than does the AMHA-TP.

The importance of the use of the AMHA-TP for screening is shown by the fact that the VDRL test alone failed to indicate reactivity in 4226 (56.54%) of 7474 sera which were reactive to both the AMHA-TP and the FTA-ABS test and thus came from persons who were infected at one time with *T pallidum*. The significance of detecting specific antibodies in the sera of these patients has been emphasised by the serological results of the 53 selected patients already mentioned.

REACTIVITY OF CSF SAMPLES

Little is known about the comparative reactivity of serum and cerebrospinal fluid (CSF) to the haemagglutination assay. Specimens from 238 donors were examined. The AMHA-TP (micro-method) was performed using serum and the TPHA (macro-method) using CSF. There was no difference in the method of examination of serum and CSF by the other two tests (FTA-ABS and VDRL).

COMPARATIVE EVALUATION

A comparative evaluation of the reactivity to the three tests on serum and CSF is summarised in Table III. The sera of two patients were non-reactive but the CSF was reactive to the AMHA-TP test. Both patients had clinical features of tabes dorsalis, one with markedly increased CSF protein (0.83 g/l) and a normal cell count (1/ml), the other with slightly

TABLE III Comparative evaluation of serological test results for serum and cerebrospinal fluid

Reactivity of serum to	Reactivity of CSF to											
	TPHA				VDRL				FTA-ABS			
	-		+		Total		-		+		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
AMHA-TP			2	0.84	2	0.84						
-												
+	84	35.29	152	63.87	236	99.16						
Total	84	35.29	154	64.71	238	100.00						
VDRL												
- (+/-)							138	57.98			138	57.98
+							85	35.72	15	6.3	100	42.02
Total							223	93.70	15	6.3	238	100.00
FTA-ABS												
-									23	9.66	23	9.66
+							144	60.51	71	29.83	215	90.34
Total							167	70.17	71	29.83	238	100.00

+ Reactive; +/- weakly reactive; - non-reactive

increased protein and a cell count of 4/ml. The serum and CSF of the first patient were both reactive to the haemagglutination test at a dilution of 1/40, which is a reactive result for CSF but a non-reactive result for serum. Both the serum and the CSF were non-reactive to the FTA-ABS and VDRL tests. The serum of the second patient was non-reactive to the haemagglutination assay and to the VDRL test; the FTA-ABS test result was borderline on several different attempts with material from the same sample whereas the CSF was reactive to the haemagglutination tests at a dilution of 1/40 and non-reactive to the FTA-ABS and VDRL tests.

The antibody titre, as detected by the AMHA-TP, was equally high in CSF and serum, which indicates that these antibodies have not necessarily come from the serum since the protein values did not indicate a breakdown of the blood-CSF barrier; thus they may have been produced within the cerebrospinal system.

Of the donors who were sero-reactive to the haemagglutination assay, 65.3% were also CSF-reactive to the same test. The figures for the FTA-ABS and VDRL tests were 33% and 15% respectively. This seems to indicate that the haemagglutination method is twice as sensitive as the FTA-ABS test and four times as sensitive as the VDRL test for CSF.

In a comparative quantitative evaluation of the results of the haemagglutination assay higher titres occurred in CSF in 11 (4.62%) of 238 samples and in serum in 224 (94.12%) of 238 samples. The VDRL titres were all higher in serum than in CSF; this suggests that the haemagglutination assay may sometimes be more sensitive in CSF than in serum when quantitatively evaluated. Further research is however necessary to improve the technique and reduce the margin of error.

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